

Descriptions of Additional Supplementary Files

Supplementary Movie 1

Description: mPTSPEX26 membrane reporter reveals abundant inner membranes in peroxisomes. Imaging cotyledons of 4-day-old seedlings expressing mNG-mPTSPEX26 (green) and mRuby3-PTS1 (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells reveals ILVs in peroxisomes. Images were acquired using a 1.04 μm interval with a 2.08 sec scan time. Scale bar is 20 μm .

Supplementary Movie 2

Description: mPTSPEX22 membrane reporter reveals abundant inner membranes in peroxisomes. Imaging cotyledons of 3-day-old seedlings expressing mPTSPEX22-mNG (green) and mRuby3-PTS1 (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells reveals ILVs in peroxisomes. Images were acquired using a 0.25 μm interval with a 1.56 sec scan time. Scale bar is 20 μm .

Supplementary Movie 3

Description: Multivesicular mPTS-labeled structures are not endosomes. Imaging cotyledons of 4-day-old seedlings expressing mNGmPTSPEX26 (green) and stained with FM4-64 to mark the plasma membrane and endosomes (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells. mNG-mPTSPEX26-labeled membranes lack FM4-64 and are distinct from endosomes. Images were acquired using a 0.38 μm interval with a 1.56 sec scan time. Scale bar is 20 μm .

Supplementary Movie 4

Description: Multivesicular mPTS-labeled structures are not autophagosomes. Imaging cotyledons of 4-day-old atg7-4 seedlings expressing mNG-mPTSPEX26 (green) and mRuby3-PTS1 (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells reveals ILVs in peroxisomes. Images were acquired using a 1.04 μm interval with a 2.08 sec scan time. Scale bar is 20 μm .

Supplementary Movie 5

Description: Peroxisomal ILVs are visible with a luminal reporter. Time-lapse imaging of hypocotyl cells of 3-day-old seedlings expressing the luminal mRuby3-PTS1 (magenta) reporter show peroxisomal ILVs as holes in mRuby3-PTS1 signal that appear to float freely in the peroxisome lumen. Images were acquired with a 0.5 μm interval with a 0.76 sec scan time. Scale bar is 5 μm .

Supplementary Movie 6

Description: Peroxisomes decrease in size over time as ILVs form. 3D projections of cotyledons of 4-day-old seedlings expressing mNGmPTSPEX26 (green) and mRuby3-PTS1 (magenta) imaged at high magnification with a spinning disk confocal microscope every 15 minutes for 20 hours. Large peroxisomes decrease in size over time as they acquire ILVs. Scale bar is 10 μm . The region shown in Figure 2a is boxed.

Supplementary Movie 7

Description: Peroxisomes decrease in size over time as inner membrane density increases. 3D projections of cotyledons of 4-day-old seedlings expressing mNG-mPTSPEX26 (green) and mRuby3-PTS1 (magenta) imaged at low magnification with a scanning confocal microscope every 15 minutes for 9 hours. Large peroxisomes decrease in size over time while small peroxisomes stay small. Scale bar is 20 μm . The region shown in Figure 2b is boxed.

Supplementary Movie 8

Description: Peroxisomal ILVs do not contain engulfed lipid droplets. Imaging cotyledons of 4-day-old wild-type seedlings expressing mNG-mPTSPEX26 (green) and stained with MDH to detect lipid droplets (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells. Images were acquired using a 0.1 μm interval with a 0.52 sec scan time. Scale bar is 5 μm .

Supplementary Movie 9

Description: Peroxisomal ILVs are apposed to lipid droplets. Imaging cotyledons of 4-day-old *acx2-2* seedlings expressing mNG-mPTSPEX26 (green) and stained with MDH to detect lipid droplets (magenta) from the top of the epidermal cells to the beginning of the mesodermal cells. Images were acquired using a 0.1 μm interval with a 0.76 sec scan time. Scale bar is 5 μm . Supplementary Movie 10. Peroxisomal ILVs may have distinct membrane protein domains. Imaging cotyledon epidermal cells of 4-day-old seedlings expressing mNG-mPTSPEX26 (green) and mRuby3-PTS1 (magenta) reveals